-Please amend the paragraph at page 44, beginning at line 21 through page 45, line 6.

Subsequently, each of the resultant promoter-appended amplicons was used as template in two separate transcription reactions. The T7 R&DNA polymerase (Epicentre, Madison, WI) was used to prepare transcripts that incorporate dCMP or dUMP instead of respectively CMP and UMP (referred to as the dC- and dU-transcripts). The transcription reactions were carried out as described in Example 2, except that each rNTP was present at 2mM and incubation was performed overnight at 37°C. The four full-length T7-transcripts were purified by annealing with a biotinylated oligonucleotide that matches with the transcript 3'-end (*i.e.* the biotinylated form of either the forward or the reverse PCR primer used in the first amplification step) and subsequent capture onto streptavidin microparticles. After extensive washing with $(NH_4)_3$-citrate, the transcripts were eluted. The beads were resuspended in 3 µl of water and kept at 90°C for 2 min, immediately followed by collection of the beads with the magnet and transfer of the supernatant to a fresh tube. Then, the obtained amplified target nucleic acids were digested to completion by the addition of 1 µl of 100 mM $(NH_4)_3$-citrate containing RNase-A. Finally, the reaction products were analyzed by MALDI-TOF-MS.

REMARKS

I. The Amendments to the Specification

The amendments to the specification were made to correct typographical errors found in the detailed description. Support for the amendment can be found in the priority document, U.S. Provisional Patent Application No. 60/131,984, at pages 18-20.

Pursuant to 37 C.F.R. §1.121, a marked-up version of the amendment to the specification made herein is attached as Appendix A.

The amendment includes no new matter.

The Detailed Description section of the specification contains several experimental Examples which are included to more clearly set forth exemplary embodiments. These Examples contain units of measurement for both solid (e.g. gram (g), milligram (mg) and

microgram (μg)) and liquid reagents (e.g. liter (L), milliliter (ml), and microliter (μl)) which are commonly used by those skilled in the art and were used by the Applicants to carry out the invention.

Subsequent to filing the application, the Applicants observed several instances in the specification where the abbreviation "μg" or "μl" placed after a numeric value is incomplete and only a "-g" or "-l" is present. As is readily apparent to one of skill in the art, an "l" does not symbolize any specific amount in and of itself as "one liter" would be depicted as "1 L." Thus, it appears that the Greek symbol for micro, μ, was inadvertently omitted in several places in the specification as a result of word-processing formatting error. The specification has been amended to correct these typographical errors in Example 2 and Example 3 of the specification.

The amendments find support from the priority application, U.S. Provisional Patent Application number 60/131,984 and International Patent Application No. WO00/66771. Example 2 of the U.S. priority document, beginning on page 18, cites Fu, D-J. *et al., (Nature Biotechnology* 16:381-384. 1998, attached herewith as Appendix B) (hereinafter Fu *et al.*) as a source for information about the Sanger chain extension method as well as the origin of the PCR protocol that is described in Example 2 of the present application (page 41, lines 11 through 15).

Fu *et al.* follows a protocol for a PCR reaction in a 50 μl volume that includes 1 unit (U) of Taq, and 0.2 mM dNTPs, corresponding to the PCR reaction disclosed at page 41, lines 12 and 13 which describes "a total volume of 50 _l using 12.5 pmol each of the forward and reverse primer, 200 _M of each dNTP, 0.25 _l U Taq (5U/_l; Promega, Madison, WI)." In light of the citation to Fu *et al.* in the priority document, and reference by both Fu *et al.* and the present specification to techniques commonly used in the art (*e.g.* PCR), it is readily discernible by one of skill in the art that the sentence in the current application should refer to a 50 μl volume with 200 μM dNTP and 0.25 μl Taq (5U/μl; Promega, Madison, WI).

Basic mathematical conversions indicate that the 0.2 mM dNTP in Fu *et al.* equals the 200 μM dNTP used in the present invention. Additionally, Promega Corp. discloses in its product catalog (attached herewith as Appendix C) that Taq polymerase is available for purchase

at a concentration of 5U/µl, which implies that 5U/µl x 0.25 µl = 1.25 U Taq, approximately equivalent to the 1 U Taq used by Fu *et al.*

Example 2 of the priority document refers to transcription reactions run in a 50 µl volume, exactly as it is found in the text of the present specification at page 41, line 25-26, which reads "the transcription reactions were run in a 50 _l volume." Thus, this unit of measurement is appropriately amended to read 50 µl based on the disclosure in the priority document.

The priority document also refers to Fu *et al.* in the description of isolating strepatavidin coated magnetic dynabeads, similar to the method disclosed on page 42, lines 9 through 21 of the present specification. While Fu *et al.* does not disclose the exact reagent quantities utilized by the present method likely due to optimization requirements of each experiment, the relative order of magnitude of the volumes and enzyme amounts is similar, e.g the Materials and Methods of Fu *et al.* describes a method of MALDI-TOF MS using amounts as little as 0.3 µl. As such, it is readily apparent to one of skill in the art that the specification refers to amounts in the µl realm as is utilized by Fu *et al.*

The U.S. priority document describes the analysis of wild-type RNase-T1 sequence utilizing primers which incorporate either the T3 or T7 promoter site, and cites both Hacia *et al.*, (*Nucleic Acids Res.* 26:4975-82. 1998, attached herewith as Appendix D) (USSN 60/131,984, page 19, line 16) (hereinafter "Hacia *et al.*") and Hahner *et al.*, (*Nucleic Acids Res.* 25:1957-64. 1997) (USSN 60/131,984, page 20, line 16)(hereinafter "Hahner *et al.*", attached herewith as Appendix E) as references to UTP replacement with m^5UTP and MALDI-MS protocols, respectively. While Hacia *et al.* and Hahner *et al.* do not disclose the exact reagent quantities utilized by the present method, the order of magnitude of the volumes and enzyme amounts is similar to the amounts utilized in the present invention. For example, the Materials and Methods of Hahner *et al.* describes a method wherein synthesized RNA transcripts are digested with µg/µl quantities of RNase and isolated using streptavidin coated beads wherein the beads are eluted in a 6 µl volume and subsequently resuspended in 2 µl of water. This protocol is similar to the one described in the specification where in the streptavidin microbeads are suspended in 3 "-l" water and digested by addition of 1 "-l" of an RNase containing solution. It follows that one of skill in the art would understand the specification to refer to microbeads suspended in a 3 µl volume and

1 µl of an RNase containing solution added to the mixture, as in Hahner *et al.* As such, the Applicant submits that the inclusion of the term µl to the specification does not constitute new matter.

Further, the priority document USSN 60/131,984 states at page 20 line 18 that all of the references cited herein are incorporated by reference.

II. Conclusion

As described above, the amendment to the present specification is made to correct typographical errors that were inadvertently omitted but have support in the priority documents. The present application relies on U.S. Provisional Application No. 60/131,984 and International Patent Application No. WO00/66771 for priority which cite references that disclose methods similar to the description in the specification. Each priority document specifically states that these citations are incorporated by reference.

Utilizing the protocols referenced in the cited art and the well-established knowledge of those skilled in the art of molecular biology, it is readily apparent that the present specification contains numerous instances of units of measurement containing the Greek symbol for micro-, µ, but the symbol was inadvertently omitted from the current specification. The inclusion of the symbol herein is based on the disclosure in the priority documents wherein the symbols were incorporated by reference.

The Applicant's submit that the amendments to the specification add no new matter to the application, but merely clarify the protocols referred to in the U.S. provisional patent application from which the present application claims priority.

The Applicants believe that no fee is associated with submission of this amendment. However, should any fees be deemed necessary in connection with the filing of this document, the Commissioner is hereby authorized to deduct any such fees from Marshall, Gerstein and Borun account number 13-2855.

Respectfully submitted,

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December 10, 2002

APPENDIX A

Version marked to show changes made

In the Specification

-At page 41, line 11 through page 42, line 23:

The first step towards the sequence analysis according to the present invention involved the amplification of the 158 base-pair test sequence. The reaction was carried out in a total volume of 50 µl using 12.5 pmol each of the forward and reverse primer, 200 µM of each dNTP, 0.25 µl Taq DNA polymerase (5U/ µl; Promega, Madison, WI), 1.5 mM $MgCl_2$ and a buffer supplied with the enzyme. After an initial incubation at 94°C for 2 min, 40 cycles of the following temperature program were performed: 94°C for 30 sec, 50°C for 30 sec, and 72°C for 15 sec. The sample was kept an additional 15 min at 72°C and then chilled. The PCR reaction product was purified (High Pure PCR Product Purification Kit; Roche Diagnostics Belgium, Brussels, Belgium) and subsequently used for transcription of one specific strand. A mutant T7 RNA polymerase (T7 R&DNA™ polymerase; Epicentre, Madison, WI) with the ability to incorporate both dNTPs and rNTPs was used in the transcription reactions. In addition to a transcription with the regular ribonucleotide substrates, one reaction was performed where CTP was replaced by dCTP, while in two more separate transcriptions either dUTP or dTTP replaced UTP. The transcription reactions were run in a 50 µl volume containing: 40 mM Tris-Ac (pH 8.0), 40 mM KAc, 8 mM spermidine, 5 mM dithiothreitol, 15 mM $MgCl_2$, 1 mM of each rNTP, 5 mM of dNTP (in these cases the appropriate NTP was excluded), ~40 nM DNA template (~2 pmol), and 250 units T7 R&DNA™ polymerase. Incubation was performed at 37° C for 2 hours. After transcription, the full-length T7 *in vitro* transcripts (118 nucleotides) were purified by allowing them to anneal to the 5'-biotinylated form of the complementary reverse PCR primer (Figure 5) followed by capture of the biotinylated annealing products onto streptavidin-coated magnetic beads. To this end, 50 pmol biotinylated reverse primer was added to the transcription reactions. The mixtures were first incubated 5 min at 70°C and, subsequently, ~30 min at room temperature. Then, a slight excess of Sera-Mag™ streptavidin magnetic microparticles [Seradyn Inc, Indianapolis, IN; resuspended in 50 µl of 2M NaCl, 20 mM Tris-HCl (pH 8.0), 2 mM

EDTA] was added and the resultant mixture incubated at room temperature for 30 min with agitation. A magnetic particle collector (MPC; Dynal, Oslo, Norway) was used to collect the beads, remove the supernatant and, subsequently, to wash the beads three times with 100 µl 100 mM $(NH_4)_3$-citrate. The beads were finally resuspended in 3 µl 25 mM $(NH_4)_3$-citrate containing 0.5 µg bovine pancreas RNase-A (50U/mg; Roche Diagnostics Belgium, Brussels, Belgium) and incubated at room temperature for about 30 min to digest the transcripts to completion. 1 µl of this RNase reaction was removed and added to 5 µl matrix solution. This 1:1 acetonitrile:H_2O matrix solution is saturated with 3-hydroxypicolinic acid (~100 mg/ml), and further contains 25 mM $(NH_4)_3$-citrate, (occasionally) 2 pmol/µl of an oligonucleotide serving as an internal standard, and cation-exchange beads in $(NH_4)^+$-form (Dowex 50W-X2; Sigma, Saint-Louis, MO) to minimize the presence of sodium and potassium adducts. After incubating the mixture at room temperature for 15 min, 1 µl was put on the sample plate and allowed to dry. Mass spectra were collected using a Reflex III mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany).

-At page 44, beginning at line 21

Subsequently, each of the resultant promoter-appended amplicons was used as template in two separate transcription reactions. The T7 R&DNA polymerase (Epicentre, Madison, WI) was used to prepare transcripts that incorporate dCMP or dUMP instead of respectively CMP and UMP (referred to as the dC- and dU-transcripts). The transcription reactions were carried out as described in Example 2, except that each rNTP was present at 2mM and incubation was performed overnight at 37°C. The four full-length T7-transcripts were purified by annealing with a biotinylated oligonucleotide that matches with the transcript 3'-end (*i.e.* the biotinylated form of either the forward or the reverse PCR primer used in the first amplification step) and subsequent capture onto streptavidin microparticles. After extensive washing with $(NH_4)_3$-citrate, the transcripts were eluted. The beads were resuspended in 3 µl of water and kept at 90°C for 2 min, immediately followed by collection of the beads with the magnet and transfer of the supernatant to a fresh tube. Then, the obtained amplified target nucleic acids were digested to completion by the addition of 1 µl of 100 mM $(NH_4)_3$-citrate containing RNase-A. Finally, the reaction products were analyzed by MALDI-TOF-MS.